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Fen Zhang,[1,2,5] Tao Zuo,[1,2,5] Yating Wan,[1,2] Zhilu Xu,[1,2] Chunpan Cheung,[1,2] Amy Y. Li,[1] Wenyi Zhu,[1,2] Whitney Tang,[1,2] Paul K.S. Chan,[3,4] Francis K.L. Chan,[1,2,4] and Siew C. Ng[1,2,4,*]

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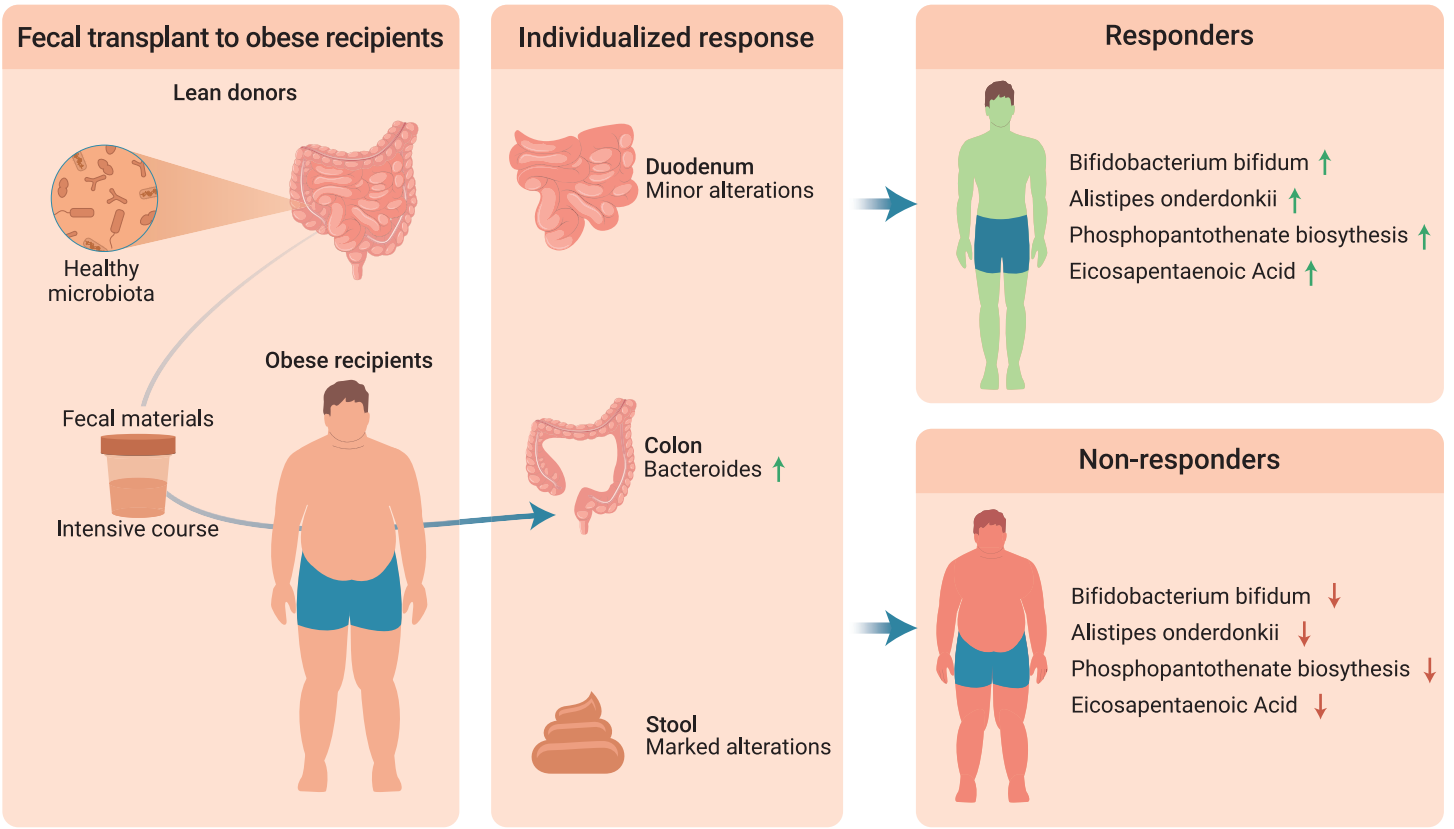
## GRAPHICAL ABSTRACT




## PUBLIC SUMMARY

- Fecal microbiota transplantation (FMT) led to individualized response in obese subjects
- FMT was less influential in shaping microbiota of the small intestine
- Specific bacteria and metabolic pathways were associated with weight loss after FMT

Report



# Multi-omic analyses identify mucosa bacteria and fecal metabolites associated with weight loss after fecal microbiota transplantation

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Fecal microbiota transplantation (FMT) has shown promising results in animal models of obesity, while results in human studies are inconsistent. We aimed to determine factors associated with weight loss after FMT in nine obese subjects using serial multi-omics analysis of the fecal and mucosal microbiome. The mucosal microbiome, fecal microbiome, and fecal metabolome showed individual clustering in each subject after FMT. The colonic microbiome in patients showed more marked variance after FMT compared with the duodenal microbiome, characterized by an increased relative abundance of *Bacteroides*. Subjects who lost weight after FMT sustained enrichment of *Bifidobacterium bifidum and Alistipes onderdonkii* in the duodenal, colonic mucosal, and fecal microbiome and increased levels of phosphopantothenate biosynthesis and fecal metabolite eicosapentaenoic acid (EPA), compared with those without weight loss. Fecal levels of amino acid metabolism-associated were positively correlated with the fecal abundance of *B. bifidum*, and fatty acid metabolism-associated metabolites showed positive correlations with *A. onderdonkii*. We report for the first time the individualized response of fecal and mucosa microbiome to FMT in obese subjects and highlight that FMT is less capable of shaping the small intestine microbiota. These findings contribute to personalized microbe-based therapies for obesity.

## INTRODUCTION

Obesity is a global pandemic with immense health consequences for individuals and societies.[1,2] Multiple factors, including genetic predispositions,[3,4] mode of delivery,[5,6] breastfeeding,[7] exercises,[8] and diet,[9] have been shown to affect the risk of development of obesity. In the last decade, the microbiome field has made tremendous progress in identifying a link between intestinal dysbiosis and obesity.[1,10] Mounting evidence points to the role of gut microbiota in obesity pathogenesis, leading to the surge of microbiome-based interventions in obesity therapeutics.

Fecal microbiota transplantation (FMT) has been considered a potential therapy for obesity and related metabolic disease.[11–13] A few randomized controlled studies showed that microbiota infusion from lean donors to subjects with metabolic syndrome was associated with improved insulin sensitivities[12] and decreased HbA1c level[11] in obese patients with type 2 diabetes (T2D). However, these studies reported no significant change in body weight after FMT intervention. To date, the underlying microbial basis, predictors of therapeutic outcome, and the ultimate active constituent(s) of FMT mediating benefit in patients with obesity remain largely unknown.

Each segment of the human intestine has specific physiological functions and distinct microbiota.[14] The small intestine is a major site for nutrient digestion and absorption, and the colon performs water extraction, nutrient fermentation, and stool formation,[15] which play an essential role in weight regulation and insulin resistance.[16,17] Several studies have demonstrated changes in stool microbiota of obese recipients after FMT intervention, including increased gut microbial diversity and butyrate-producing bacteria.[11,12,18–20] However, these FMT studies were all built on the fecal microbiome. Whether and how FMT modifies the mucosal microbiome remain largely unknown.

We hypothesize that antibiotic preparation followed by an intensive course of FMT can increase the engraftment of gut microbiota from donors to recipients, which would be associated with weight loss in obesity. Here, we conducted an open-label, intensive FMT pilot study on obese individuals, in which patients received a 1-month course of FMT following a 3-day preparation treatment of a cocktail of antibiotics to clear the "obese" microbiome. We investigated the longitudinal dynamics of duodenal, colonic, and fecal microbiota in obese recipients after FMT. We further performed a multi-omics analysis combining fecal metagenomics, metatranscriptomics, and metabolomics to characterize functional changes associated with weight loss.

## RESULTS

### Obese subjects and FMT regimes

Nine obese subjects with a body mass index (BMI) ranging from 31.9 to 41.5 kg/m$^2$ were recruited (Table S1). Each subject received antibiotic preparations for 3 days, followed by an intensive course of FMT daily for 5 consecutive days each week (5 days on and 2 days off) for 4 weeks. After FMT, they were followed up for 12 weeks (Figure 1A). Serial fecal samples were collected at weeks 0, 1, 2, 3, 4, 6, 8, and 12 for metagenomic, metatranscriptomic, and metabolomic profiling. In addition, duodenum and colon biopsies were taken from subjects during the 1-month FMT for 16s rRNA profiling of the mucosal microbiome (Figure 1A). Each recipient received FMT from a single lean donor (subjects 1–4 from donor 1; subjects 5 and 6 from donor 2; subjects 7, 8, and 9 from donors 3, 4, 5, respectively) (Figure 1A).

After intensive FMT intervention, nine subjects showed variable weight loss at 12 weeks of follow-up (2.12 ± 1.87 kg at week 12, means ± SEs, Figure 1B). Subjects 5, 6, and 7 had sustained a >4 kg weight loss after FMT at week 12. Subject 7 had a maximum weight loss of 8.1 kg at week 12. Subjects 1 and 8 showed a weight reduction of 4 kg after cessation of FMT, which rebounded slightly afterward. The clinical outcome suggests that intensive FMT leads to a modest and variable weight reduction in subjects with obesity.

### FMT led to alteration of *Bacteroides* in the colonic mucosal microbiome in recipients

We conducted taxonomic analyses on the duodenal and colonic mucosal microbiome via 16S rDNA profiling. At the bacterial community level, the post-FMT mucosal microbiome clustered with respect to each recipient, both for the duodenal and colonic microbiome (Figure 2A). Subject individuality accounted for 32.4% of the duodenal microbiome variance (based on Bray-Curtis dissimilarities between the microbiome of different subjects, permutational multivariate ANOVA [PERMANOVA] test, p = 0.001), whereas the post-FMT time point explained only 1.5% of the variance (PERMANOVA test, p = 0.165). Similarly, subject individuality accounted for 33.2% of the colonic microbiome variance (based on Bray-Curtis dissimilarities, PERMANOVA test, p = 0.001), whereas time point explained only 3.5% of the variance (PERMANOVA test, p = 0.161). These data suggest that the mucosal microbiome is highly individualized and that interindividual configurational differences predominate over the effect of FMT in altering the mucosal microbiome in recipients. FMT resulted in more marked variance in the colonic microbiome relative to the duodenal microbiome, although the mucosal microbiome overall remained largely stable (Figure S1). At the

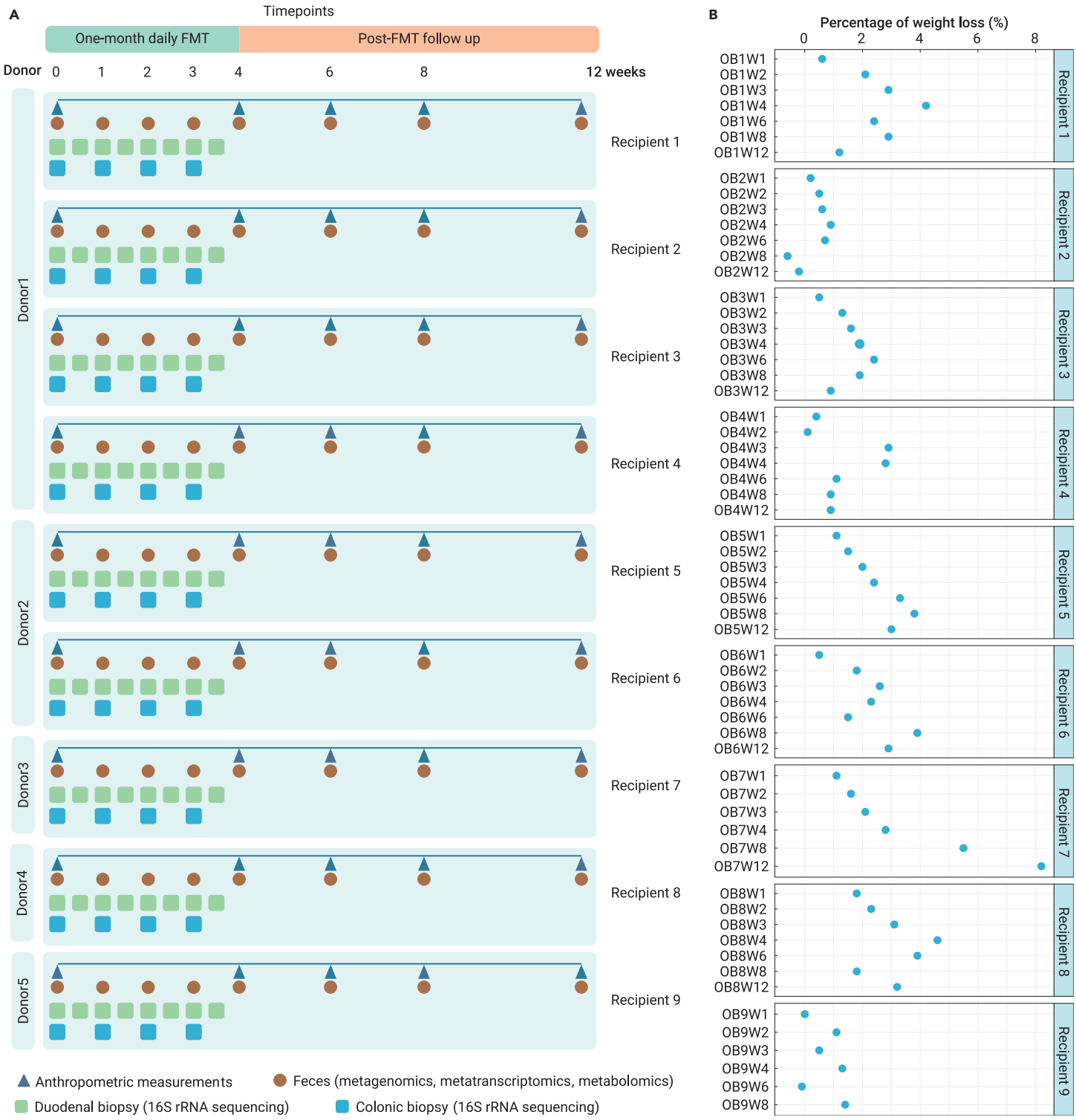


**Figure 1. Study schematic and clinical outcome** (A) Longitudinal timeline of clinical information and sample collection from 9 obese subjects treated with FMT (expressed in weeks). (B) Longitudinal body weight change of obese recipients after FMT. The y axis represents the percentage of weight loss at different time points compared with body weight at baseline. "Donor" denotes FMT donor. "FB" denotes obese subject (FMT recipient). "W" indicates the nth week since the start of FMT, where weeks 1–4 represent the 1-month daily FMT period and weeks 5–16 represent time points after FMT.

compositional level, the duodenal and colonic microbiota of recipients 1, 2, 3, 4, and 9 were dominated by *Pseudomonas* species before and after FMT, while *Massila timonae* dominated in recipients 5 and 7 before FMT and then declined after FMT in the colonic mucosa. (Figures S2A and S2B). Among all bacterial operational taxonomic units (OTUs), the genus *Bacteroides* showed the strongest correlation with the longitudinal colonic microbiome shift along MDS1 (Spearman correlation rho = 0.726, p = 2.131e−6; Figure 2B), suggesting FMT primarily drives the alteration of *Bacteroides*. It has been reported that members of the *Bacteroides* genus were depleted in obese individuals[21,22] and increased after weight loss induced by dietary intervention[22] or Roux-en-Y gastric bypass.[23,24] The restoration of *Bacteroides* in mucosa may partly account for the weight loss in obese subjects after FMT intervention.

## FMT resulted in greater alterations in the fecal microbiome than the mucosal microbiome

We next performed taxonomic analyses on the fecal microbiome via metagenomic profiling. Compared with the mucosal microbiome, FMT led to more remarkable changes in the fecal microbiome, with a contribution of donor-derived

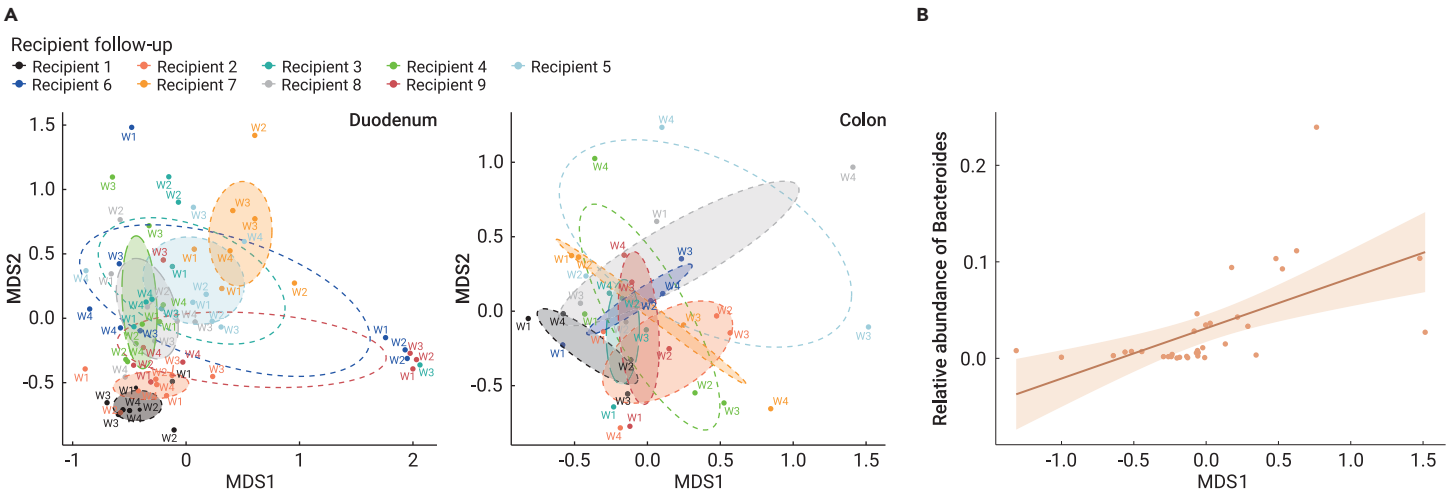


**Figure 2. Mucosal microbiome shifts in obese recipients during the 1-month FMT period** (A) Microbiome community shifts in the duodenal and colonic microbiome were viewed by non-metric multidimensional scaling (NMDS) plot based upon Bray-Curtis dissimilarities. (B) Spearman correlation between the genus *Bacteroides* with longitudinal colonic microbiome shift along MDS1. The MDS1 value was calculated based on Bray-Curtis dissimilarities. "W" and "D" represent time points since the start of FMT: "W" indicates the nth week since the date of the first FMT; ""D" denotes the nth day within the indicated week. "W1D1" denotes the subject baseline, and the biopsy sample was collected before FMT.

species in recipients ranging from 0.04% to 68% (9.4% ± 12.8%, means ± SEs; Figure S3). Fecal transplants from the same donor did not result in consistent colonization across different recipients, substantiated by the observation that recipients 1–4, who were transplanted with fecal matters from one single donor, showed discrepant colonization of donor-derived bacteria (Figure S3). These data suggest that host baseline features and their indigenous microbiome may affect donor bacteria engraftment to recipients. At the bacterial community level, post-FMT microbiome clustered together on the basis of recipient subjects (via Bray-Curtis dissimilarity analysis between microbiomes, PERMANOVA test, $p < 0.001$; Figure 3A). While transplants of donor 1 and donor 5 resulted in the post-FMT donor-like microbiome in recipients 1–4 and 9, donors 2–4 did not induce the microbiome proximity to donors in recipients 5–8 (Figure 3A). These findings highlight the microbiome individuality in response to FMT in obese recipients. In addition, before FMT, the microbiome richness of obese recipients was significantly higher than donors (paired t test, $p = 0.033$; Figures 3B and 3D), which decreased after FMT (paired t test, $p = 0.058$), indicating a post-FMT species loss in addition to species acquirement during FMT. Meanwhile, these obese subjects displayed heterogeneous post-FMT variations in microbiome diversity, with no significant differences before and after FMT (Figures 3C and 3E).

We then explored how FMT altered the taxonomy of species composition in obese recipients. These nine recipients had different dominant species after FMT, where *Bifidobacterium adolescentis* predominated in the post-FMT samples of recipients 4–6 and recipients 8–9. *B. bifidum* was increased after FMT and dominated in the post-FMT samples of recipients 5–7, who achieved the most significant weight loss after FMT, while it was absent in other FMT recipients (Figure 4A). Interestingly, only donors 2 and 3, who were recipients 5–7′ donors, showed the presence of *B. bifidum*, compared with other donors. *B. bifidum* has been reported to have anti-obesity effects and to suppress lipid deposition in mice.[25] Our result suggests that the presence of *B. bifidum* in donors may be associated with the engraftment of this bacteria and weight loss in their respective recipients. A regression analysis via least absolute shrinkage and selection operator (LASSO) on the post-FMT fecal microbiome and weight loss identified a number of bacterial species associated with weight change. In addition to *B. bifidum*, increased relative abundances of *Bacteriodes vulgatus* and *Alistipes onderdonkii* showed the strongest correlation with weight loss in recipients after FMT ($p < 0.05$; Figures 4B and 4C). *Bacteriodes vulgatus* was substantially increased after FMT in recipients 5–7, who had the most weight reduction, which may be partly attributable to the high presence of *Bacteriodes vulgatus* in their corresponding donors (Figure 4B). *A. onderdonkii* was substantially increased after FMT in recipient 1 and recipient 5, who achieved weight loss after FMT, exhibiting markedly high relative abundances versus that in other recipients. However, the abundance of *A. onderdonkii* in donors was not associated with the engraftment of this taxon and weight loss in recipients after FMT (Figure 4C). We further examined the relative abundance of *B. bifidum*, *A. onderdonkii*, and *B. vulgatus* in the duodenal and colonic microbiome. In line with the fecal bacteria data, *B. bifidum* showed prominent existence in the mucosal samples (in both duodenum and colon) of recipients 5–7 during the 1-month FMT (Figures S4A and S4B), while *A. onderdonkii* was consistently highly represented in recipients 6 and 7 and steadily increased in recipient 1 in the colonic microbiome (Figures S4C and S4D). Altogether, these data suggest that the increased abundance of the species *B. bifidum*, *Bacteriodes vulgatus*, and *A. onderdonkii* after FMT in obese subjects may be associated with weight loss.

## FMT was associated with an altered microbial function of phosphopantothenate biosynthesis

We then explored whether FMT was associated with altered gut microbiome functionality in obese subjects. We studied fecal microbiome functionality at the microbial DNA level via *HUMAnN2* and correlated the abundance profile of metabolic pathways (MetaCyc) with weight change after FMT via LASSO analysis. Change in metabolic pathways correlated with weight change after FMT was shown in Figure 5A and Table S2. Three metabolic pathways, phosphopantothenate biosynthesis I, glycolysis III (from glucose), and superpathway of L-serine and glycine biosynthesis, were the most abundant in obese subjects and were positively correlated with weight loss. These three pathways increased significantly after FMT. Underrepresentation of these pathways has been reported to be associated with metabolic disorders, including obesity and type 2 diabetes.[26–28] The increase in abundance of these metabolic pathways after FMT may be associated with weight loss in obese subjects.

As measures of the microbial gene expression (at the RNA level) is more informative than metagenomic profiles of functional potential (at the DNA level),[29,30] we next performed shotgun metatranscriptomic sequencing on a subset of FMT subjects (recipients 1, 2, 5, and 7) to understand microbial functional activity and to verify our DNA-level findings. Similarly, LASSO analysis of metatranscriptomic profiles and post-FMT weight change discerned metabolic pathways (at the RNA level) were significantly associated with weight loss (Figure 5B; Table S3). In line with the microbial gene content finding, phosphopantothenate biosynthesis I was the most abundant pathway positively associated with weight loss after FMT (Figure 5B). The microbial RNA expression of the phosphopantothenate biosynthesis I pathway was increased after FMT in recipients 5 and 7, who achieved the most significant body weight loss after FMT, where *Bacteroides vulgatus* contributed the most to this microbial functional activity (Figure 5C). Phosphopantothenate can be related to the reduced resistance to insulin and activation of lipolysis in serum and adipose tissue.[26]

To substantiate the associations between weight loss and the bacterial species *B. bifidum*, *Bacteriodes vulgatus*, and *A. onderdonkii* at the taxonomic level (Figure 4), we further investigated the functional potential (at the DNA level) and activity (at the RNA level) of these three bacteria species after FMT administration. The abundance of functional pathways of *B. bifidum* and *Bacteriodes vulgatus* were higher in FMT donors and recipients 5–7, who showed the most significant body weight loss after FMT, compared to that in other FMT recipients

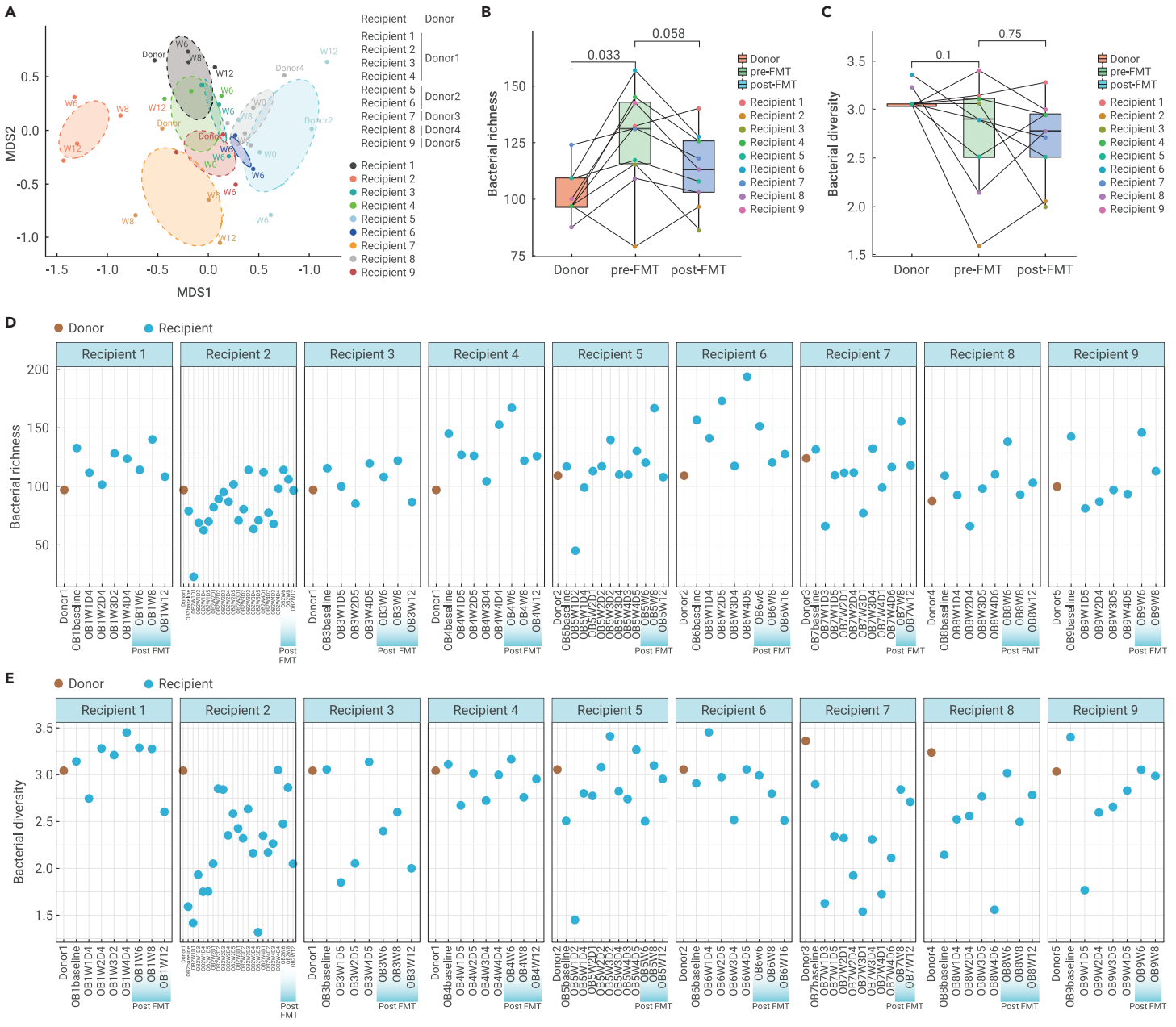


**Figure 3. Post-FMT alterations in the fecal microbiome beta and alpha diversity of obese recipients** (A) Microbiome community alterations after FMT, viewed by NMDS plot based upon Bray-Curtis dissimilarities. (B and D) Fecal microbiome Chao1 richness in FMT recipients and their corresponding donors at baseline. (C and E) Fecal microbiome Shannon diversity in FMT recipients and their corresponding donors at baseline. Comparisons of the microbiome richness and diversity between donors, pre-FMT, and post-FMT last follow-up were statistically tested by paired Wilcoxon signed rank test, *p < 0.05. "OB" denotes obese subject (FMT recipient). "Donor" denotes FMT donor. "baseline" denotes subject baseline, and the samples were collected before antibiotics treatment. "W" and "D" represent time points after FMT: "W" indicates the nth week since the start of FMT, where weeks 1–4 represent the 1-month daily FMT period and weeks 5–16 represent time points after FMT; "D" denotes the nth day within the indicated week.

(Figure S5A). The abundance of functional pathways of *A. onderdonkii* was higher in post-FMT recipients 1 and 5 than in other FMT recipients (Figure S5A). Analogously, at the metatranscriptional level, the functional activities of *B. bifidum* and *Bacteriodes vulgatus* were increased and highly present in recipients 5 and 7, who showed the most significant weight loss after FMT, relative to recipients 1 and 2 (Figure 5B). In addition, the metabolic functions of *A. onderdonkii* were actively present in recipients 1 and 5, who achieved weight loss after FMT, while they were absent in recipient 2, who showed weight gain after FMT (Figure S5B).

## FMT resulted in metabolomic alteration in obese subjects

We next performed the widely targeted metabolomics analysis of fecal samples to explore how FMT alters fecal metabolites in association with weight change. The metabolic profiles of obese subjects at baseline differed significantly from that of donors, as shown by the non-metric multidimensional scaling (NMDS) analysis, whereby donor profiles clustered away from those of obese recipients (PERMANOVA test, p = 0.006; Figure 6A). The metabolic profiles of post-FMT recipients clustered with respect to subjects, suggestive of individuality in the metabolic response to FMT (Figure 6A). In accordance, subject individuality accounted for 31.8% of the compositional variance in fecal metabolome (PERMANOVA test, p = 0.001). To further dissect how FMT changed gut metabolome in association with post-FMT weight loss, a regression analysis via LASSO was implemented to discern metabolites associated with weight changes (Table S4). Among them, increased fecal levels of eicosapentaenoic acid (EPA) showed the strongest correlation with recipients' post-FMT weight loss. EPA was reported to have an anti-obesity effect by decreasing remnant-like particle-triglyceride, small dense low-density lipoprotein (LDL), and C-reactive protein while increasing adiponectin in humans and mice.[31,32] The higher presence of EPA in post-FMT fecal samples of recipients 5 and 7 relative to other recipients (Figure 6B) suggests that the obesity-opposing effect of FMT may be associated with its impact on modulating EPA.

We then sought to identify fecal metabolites associated with *B. bifidum*, *Bacteriodes vulgatus*, and *A. onderdonkii*. Amino acid metabolism-associated

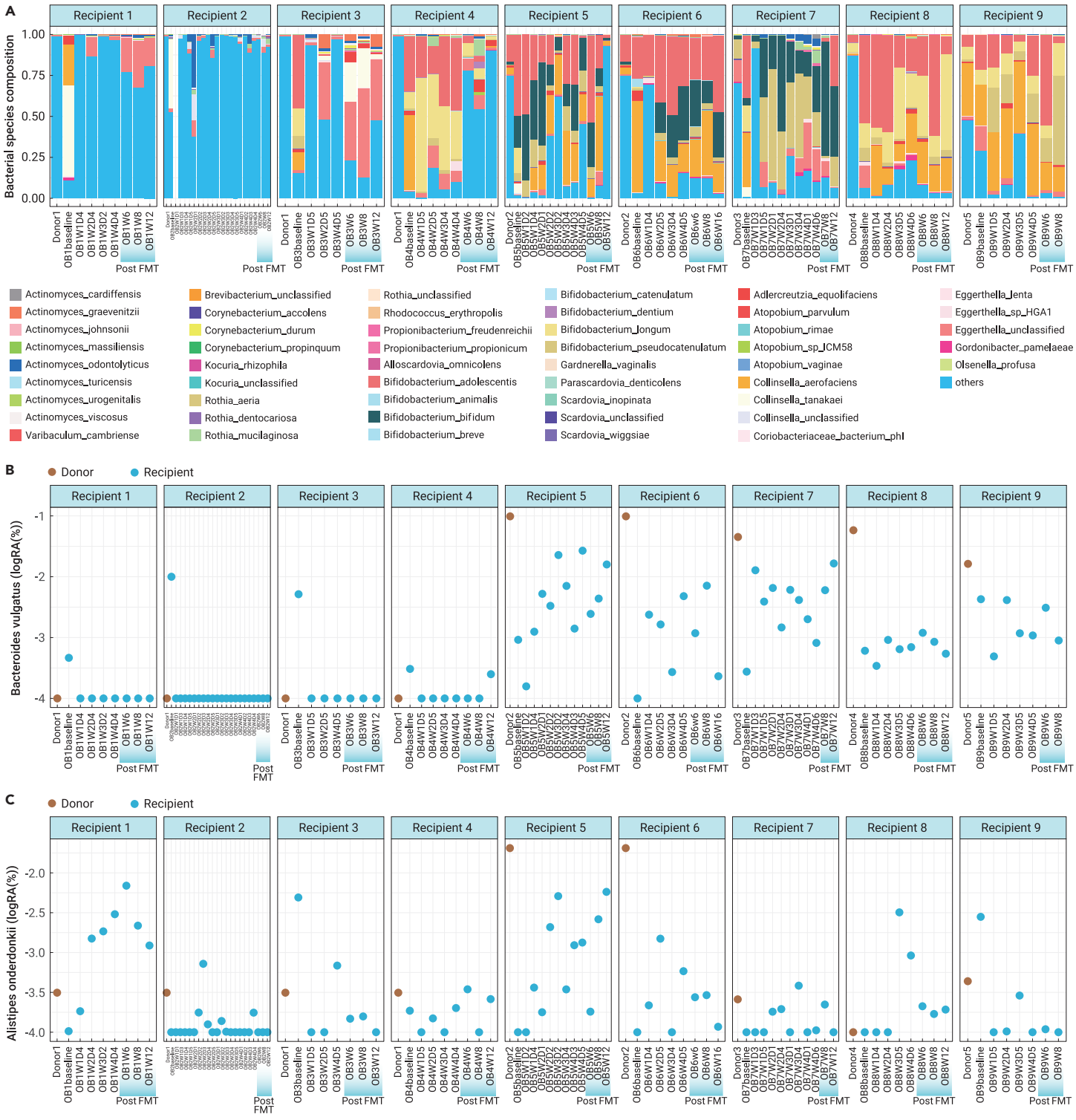


**Figure 4. Post-FMT alterations in the fecal microbiome composition of obese recipients** (A) Alterations in the fecal bacteria composition at the species level in obese recipients after FMT at different time points. Only the most abundant 50 species across all of the subjects were plotted. (B and C) The relative abundance of *Bacteriodes vulgatus* and *Alistipes onderdonkii* in the fecal microbiome of recipients at different time points after FMT.

metabolites (hexanoyl glycine, L-malic acid, L-homocitrulline, and $N^6$-acetyl-L-lysine) and EPA showed significant positive correlations with the abundance of *B. bifidum* and *Bacteriodes vulgatus*, whereas organic acid and its derivatives were positively correlated with *A. onderdonkii* (Figure 6C). Hexanoyl glycine and L-malic acid have been reported to be underrepresented in obese mice.[33,34] The concomitant changes between these fecal metabolites and the bacterial species *B. bifidum*, *Bacteriodes vulgatus*, and *A. onderdonkii* imply that FMT may modify the gut metabolic profile and microbiome community to counteract obesity. In contrast, *N*-ethylacetamide, urea, and 2-(4-hydroxyphenyl) ethanol exhibited the strongest inverse correlations with *B. bifidum*, *Bacteriodes vulgatus*, and *A. onderdonkii* (Spearman rho = −0.487, −0.40, and −0.591, respectively; Figure 6D). In favor of our finding, urea production was reported to decrease in response to weight loss in obese subjects.[35] Compared to *B. bifidum*, *Bacteriodes vulgatus* and *A. onderdonkii* showed more inverse correlations with a large number of metabolites, the majority of which belonged to organic acid, indicating that *A. onderdonkii* may mitigate obesity via tailoring its organic acid metabolites pool.

## DISCUSSION

Given the well-established causation between gut microbiota and obesity derived from animal studies,[16,36,37] attempts have been made to transplant gut

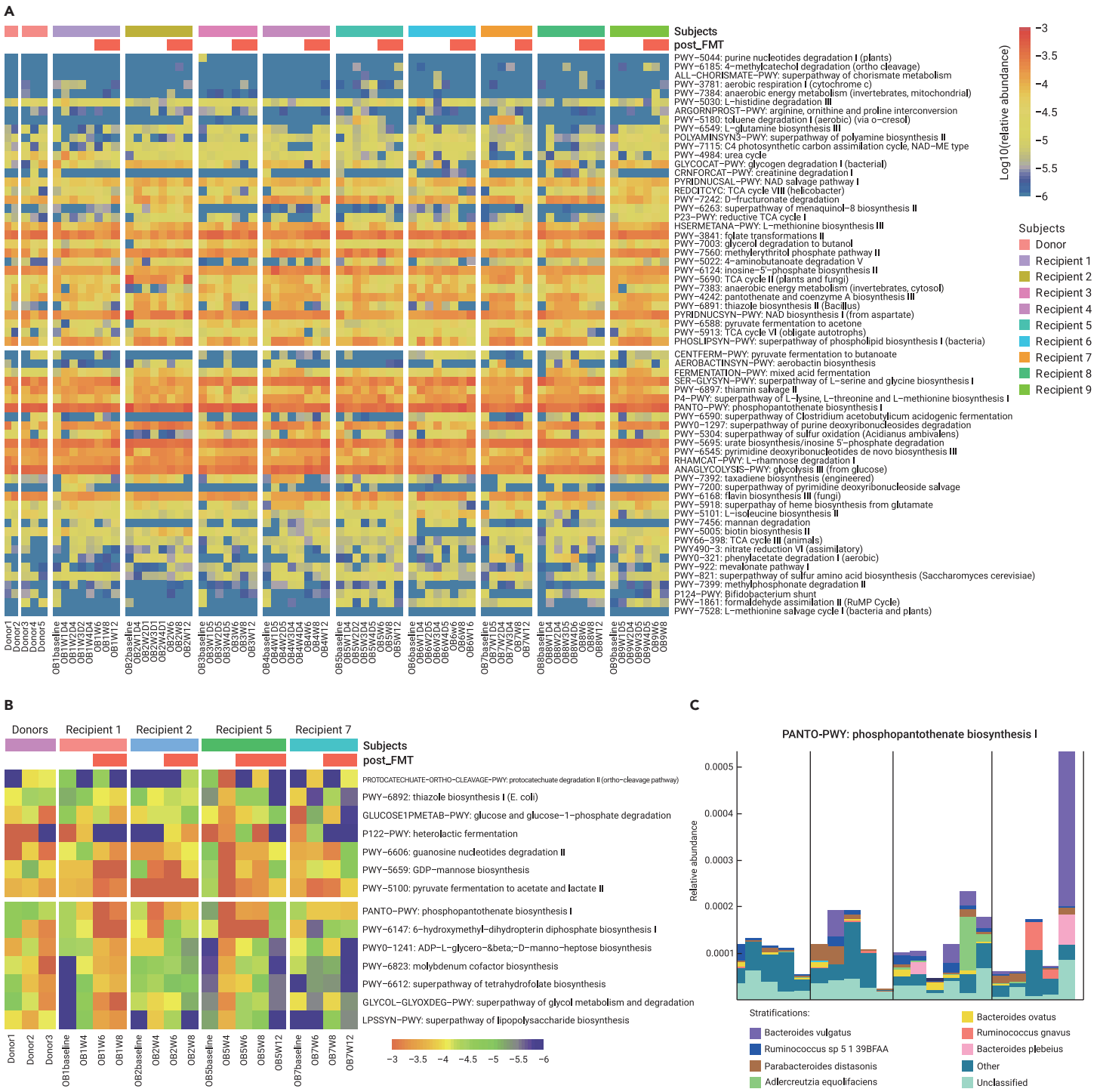


**Figure 5. Fecal microbiome functionality alterations in recipients after FMT, in association with post-FMT body weight loss** (A) Alterations in the functionality of fecal microbiome during the 1-month FMT period and after FMT at the DNA level. The abundance of metabolic functions (pathways) was profiled via HUMAnN2 based on the metagenomic dataset. (B) Alterations in the functional activity of fecal microbiome during the 1-month FMT period and after FMT at the metatranscriptional (RNA) level. The expression level of metabolic functions (pathways) was profiled via HUMAnDornorN2 based on the metatranscriptomic dataset. Metabolic pathways differentially associated with body weight change were identified via LASSO and plotted in the heatmap. Only those significant pathways included in the LASSO regression model were plotted. (C) Longitudinal expression profile of the metabolic pathway, phosphopantothenate biosynthesis I, in recipient microbiome after FMT at the metatranscriptional level. The expressional contribution to this pathway was stratified by constituent bacteria.

microbiota from lean and healthy donors into obese and metabolic syndrome recipients in human trials.[11,12,38] To our best knowledge, no human study has reported a favorable clinical outcome of significant body weight reduction by conventional FMT in obesity. Here, we reported a minor body weight loss in a subset of obese patients given 4-week intensive FMT in our clinical trial, which is consistent with the previous report by Vrieze et al.,[12] suggesting that intensively repeating FMT did not increase response in obese recipients. Such information contributes to our knowledge on developing FMT strategies to treat obesity. To date, the clinical benefits of exploiting FMT to rebuild the gut microbial ecosystem in patients with obesity and MS are not well understood. In the present study, our dense longitudinal multi-omics dataset allowed us to investigate relationships between obese phenotype and microbiome composition and functionality after FMT intervention on a systematic level.

Although Ng et al.[18] have found that obese patients acquired ≥20% of microbiota from lean donors after FMT by profiling the stool microbiome of the obese patients and lean donors, whether the changes in stool microbiota faithfully represent alterations of microbiota in different gut regions remain unclear. For the first time, our study interrogated the mucosal (both duodenal and colonic) microbiome

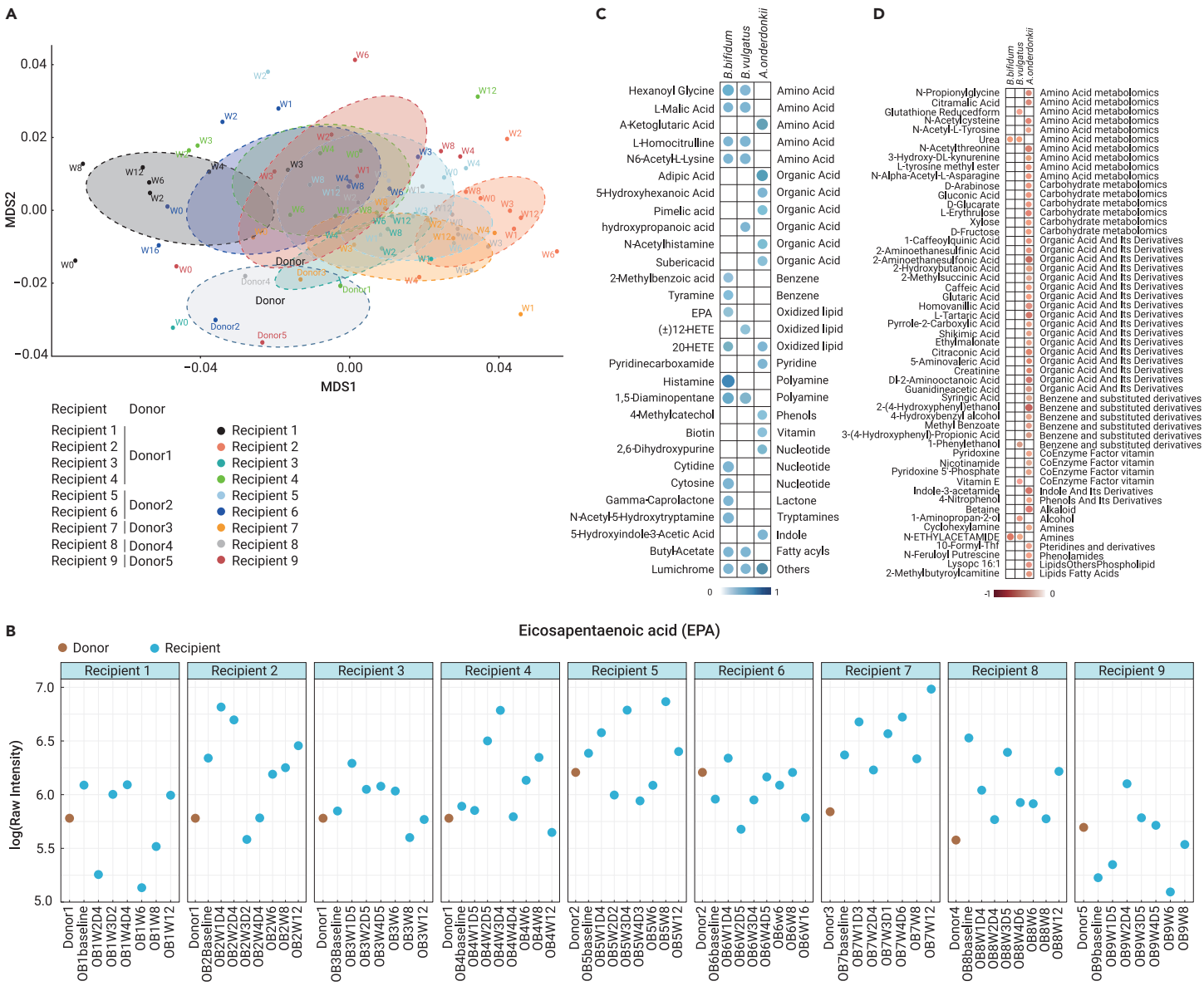


**Figure 6. Alterations of the fecal metabolome in recipients after FMT, in association with post-FMT body weight loss** (A) Alterations of fecal metabolome after FMT, viewed by NMDS plot based upon Bray-Curtis dissimilarities. (B) The abundance of eicosapentaenoic acid in the fecal microbiome of recipients at different time points after FMT. (C) Positive Spearman correlations between fecal metabolites with *Bifidobacterium bifidum*, *Bacteriodes vulgatus*, and *Alistipes onderdonkii*. (D) Negative Spearman correlations between fecal metabolites with *Bifidobacterium bifidum*, *Bacteriodes vulgatus*, and *Alistipes onderdonkii*. The dots indicate significant correlations. The size and shading of dots indicate the magnitude of the correlation, where darker shades showed higher correlations than lighter ones.

alterations in relation to FMT and disease phenotype alterations. We found that FMT has led to an increased relative abundance of *Bacteroides* in the colonic mucosal microbiome after FMT in obese subjects who lost weight, while the duodenal mucosal microbiome remained largely stable. Small intestine microbiota can regulate host digestive and absorptive adaptive responses to dietary lipids, thereby playing an important role in weight regulation and insulin resistance.[16,17] The less malleable microbiota of the small intestine in obese patients may be one of the reasons why FMT shows a poor clinical outcome in treating obesity. Our study suggests that future FMT practice in treating obesity should consider how to reshape the small intestinal microbiota to improve clinical outcomes.

In addition, our data showed that FMT led to various responses regarding phenotypic and microbiome profile alterations among obese subjects. Although donor microbial engraftment was observed across recipients, the recipients displayed personalized colonization levels and discrepant microbiome resemblances to their corresponding donor following FMT (donor 1 resulted in donor resemblance in all four recipients, whereas donors 2–5 did not; Figure 3C). In addition, unlike *Clostridium difficile* infection (CDI),[39,40] the donor-bacteria colonization ratio in recipients did not correlate with obese phenotype amelioration (body weight loss). These data suggest that certain and/or personalized gut microbiome compositional and functional changes in specific components may be crucial for improving obesity.

*Bacteroides* can actively refine the gut environment to make it more hospitable for themselves and other microorganisms.[41–43] Their ability to tolerate and reduce oxygen levels would likely aid *Bacteroides* in spreading to new hosts,[41] enabling FMT to transfer *Bacteroides* to the mucosal and fecal microbial pool of the recipient. Expansion of the genus *Bacteroides* in the colonic and fecal microbiome may partly account for the body weight loss in recipients 6–7 following FMT intervention. The significant increases in *Bacteriodes vulgatus* and *A. onderdonkii* (all members of *Bacteriodes*) in the feces and colonic mucosa recipients who lost weight after FMT imply their beneficial roles in improving host metabolic phenotypes. In addition, the hypolipidemic effects of phosphopantothenate have been reported in mice, including reduced resistance to insulin and activation of lipolysis in serum as well as adipose tissue.[26] We found that the phosphopantothenate biosynthesis I pathway (highly abundant in *Bacteroides vulgatus*) was increased in recipients who achieved weight loss after FMT on integrative metagenomic and metatranscriptomic analyses, indicating that this function may be one of the underlying mechanisms that help *Bacteroides vulgatus* combat obesity.

The increased fecal level of EPA showed the strongest correlation with recipients' post-FMT weight loss and a positive correlation with the abundance of *B. bifidum*. Bacterial polyunsaturated fatty acid (PUFA) production, including EPA, was previously believed to be limited to marine bacteria, which has been reported in recent years in members of δ-proteobacteria retrieved from soil samples.[44] However, to the best of our knowledge, no study has shown that original human bacteria can produce EPA directly. We found that *B. bifidum*, *A. onderdonkii*, and *Bacteriodes vulgatus* did not harbor enzyme genes that drove the EPA biosynthesis in prokaryotes by checking the fecal metagenomic and metatranscriptomic profiles, further suggesting that these bacteria may not be able to produce EPA. Gut microbes and their mediators drive digestive and lipid absorption through systemic control of enteroendocrine signaling and a local impact on fatty acid transport in enterocytes, therefore regulating the host lipid metabolism.[17] Supplementation of probiotics such as *B. bifidum* or FMT have been found to increase levels of EPA in breast milk, blood, and multi-organs.[45–47] Hao et al.[46] found that FMT improved T1D-disturbed gut microbiota, increased levels of n-3 PUFA docosahexaenoic acid (DHA) and EPA in blood and testicular to ameliorate spermatogenesis and semen quality. Therefore, in our study, an altered fecal level of EPA after FMT might result from changes in host lipid metabolism driven by the alterations in gut microbiota rather than EPA production by gut microbes directly, while the underlying mechanisms remain unclear.

Our study has several shortcomings. The recipients were followed up for only 12 weeks, and it remains unclear whether the alterations in weight and microbiota would remain stable or be rebounded in a long-term follow-up. In addition, the small sample size precludes generalization of our findings, and further larger sample-sized studies are needed to validate the present findings. Furthermore, we are unable to determine whether the changes in the gut microbiome directly contribute to weight loss or whether they are simply a consequence of FMT. Future mechanistic studies are warranted to delineate their significance and implications in obesity.

In conclusion, we reported for the first time the individualized response of mucosa and fecal microbiome to FMT in obese subjects and highlight the importance of reshaping the small intestinal microbiota in future FMT practice. Multi-omics analysis of fecal samples showed that specific bacteria and metabolic pathways were associated with weight loss after FMT. These findings contribute to personalized microbe-based therapies for obesity.

## MATERIAL AND METHODS

### Ethics statement

This study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (The Joint CUHK-NTEC CREC, CREC ref. no. 2018.444, Clinical Trial Registry, NCT03789461). The patients consented to participate in this study and agreed to publish the research results.

### Study subjects and design

Subjects aged 18–75 with a BMI ≥28 kg/m$^2$ and <45 kg/m$^2$ were recruited. Subjects with any of the following conditions were excluded: current pregnancy; known history or concomitant significant gastrointestinal disorders (including inflammatory bowel disease, current colorectal cancer); known history or concomitant significant food allergies; immunosuppression; known history of severe organ failure (including decompensated cirrhosis); kidney failure; epilepsy; acquired immunodeficiency syndrome; current active sepsis; known contraindications to esophagogastroduodenoscopy (OGD); use of probiotics or antibiotics in the recent 3 months; new drugs in the last 3 months that can affect metabolism or body weight; previous gastric or small intestinal surgery that alters gut anatomy such as fundoplication, gastric resection, gastric bypass, small bowel resection, ileoectomy, and colectomy; and subjects who have a confirmed current active malignancy or cancer.

A total of nine subjects were recruited. Before receiving FMT, subjects received 3 days of antibiotics consisting of vancomycin 500 mg 3 times daily, metronidazole 500 mg 3 times daily, and amoxicillin 500 mg 3 times daily to enhance the engraftment of the microbiota from FMT. All of them have received 4-week FMT infusions by OGD (1st day and 3rd day each week) and enema (2nd, 4th and 5th each week) and were followed up to week 12. Donor stools (BMI <23 kg/m$^2$) were obtained from the stool bank of the Chinese University of Hong Kong. Four recipients (recipients 1–4) were transplanted with fecal matter from the same donor (donor 1), two recipients (recipients 5–6) were transplanted with fecal matter from another donor (donor 2), while the other three recipients (recipients 7–9) were treated with another 3 different donors (donors 3, 4, and 5). Body weight was measured, and stool samples were collected at weeks 0, 1, 2, 3, 4, 6, 8, and 12. Duodenal biopsies were collected twice per week, and colonic biopsies were collected once per week during FMT treatment. Diet histories were collected in the initial assessment and follow-ups. Subjects completed a 24-h diet recall for the first 4 weeks, and thereafter, a 3-day dietary record every other week for up to 12 weeks.

### Fecal DNA extraction and metagenomic shotgun sequencing

Fecal DNA was extracted from the pellet using Maxwell RSC PureFood GMO and Authentication Kit (Promega, Madison, WI), following the manufacturer's instructions. The extracted fecal DNA was shotgun metagenomic sequenced (Illumina Novaseq 6000 with PE150 sequencing strategy by Novogene, Beijing, China) and generated an average of 52,177,661 ± 10,378,396 reads (12G) per sample.

### Fecal microbial RNA extraction and metatranscriptomic shotgun sequencing

Fecal RNA was extracted from the pellet using the Maxwell RSC simplyRNA Tissue Kit (Promega) following the manufacturer's instructions. The extracted fecal RNA was performed metatranscriptomic sequenced (Illumina Novaseq 6000 with PE150 sequencing strategy by Novogene) and generated an average of 39,722,982 ± 5,124,869 reads (10G) per sample.

### Fecal bacteria taxonomic and functionality profiling

Raw sequence reads were filtered and quality trimmed using Trimmomatic version 0.36,[48] and contaminating human reads were filtering using Kneaddata (https://bitbucket.org/biobakery/kneaddata/wiki/Home, reference database: GRCh38 p12) with default parameters. In addition, metatranscriptomic reads were filtered against the human transcriptome (hg38) and the SILVA database.[49]

Profiling of bacterial taxonomy from metagenomes of fecal DNA was extracted using MetaPhlAn2 (version 2.9) by mapping reads to clade-specific markers.[50] Functional profiling of metagenomes and metatranscriptomes was performed using humann2 version 0.11.1,[51] which included annotation of species pangenomes through Bowtie2[52] with reference to the ChocoPhlAn database, translated search of unmapped reads with DIAMOND[53] against the UniRef90 universal protein reference database,[54] and pathway collection from the generated gene list with reference to the Metacyc database.[55] Gene families and pathway abundances estimated from the data were normalized according to relative abundances of their corresponding microbial taxa reported by MetaPhlAn2.

### Mucosal bacterial DNA extraction, 16S rRNA sequencing, and taxonomic profiling

Bacterial DNA was extracted from duodenal and colonic biopsies using Maxwell RSC Tissue DNA kit (Promega) following the manufacturer's instructions.[56] The qualified DNA was sequenced on the Illumina Hiseq 2500 platform (V3-4 region, 2 × 250 bp).

Quality control and data analysis of 16S rRNA sequences were implemented in mothur (version 1.38.0), as previously described.[57] Any sequences with ambiguous bases and anything longer than 275 bp were removed and aligned against the non-redundant Greengenes database (version 13.8)[58] using the NAST algorithm. Any sequences that failed to align with the V3-4 region were discarded. The remaining sequences were trimmed to the same alignment coordinates over which they fully overlapped, followed by the removal of homopolymers and detection for the presence of chimeras by UChime. The resulting sequences were classified against the Greengenes database and annotated with deepest level taxa represented by pseudo-bootstrap confidence scores of at least 80% averaged over 1,000 iterations of the naive Bayesian classifier. Any sequences that were classified as either being originated from archaea, eukarya, chloroplasts, mitochondria, or unknown kingdoms were removed. The annotated sequences were assigned to phylotypes according to their consensus taxonomy, with which at least 80% of the sequences agreed. Closed reference OTUs sharing 97% identity were clustered as well and assigned taxonomy according to the Greengenes database. Bacteria abundance tables at different taxonomic levels were also generated.

### Fecal widely targeted metabolomics profiling

A total of 50 mg per fecal sample was homogenized with 500 μL ice-cold methanol/water (70%, v/v). Then, the mixture was vortexed for 3 min, sonicated for 10 min in an ice-water bath, and vortexed for 1 min. The supernatant was collected by centrifuging at 12,000 rpm at °C for 10 min and was analyzed using a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) system (ultraperformance LC [UPLC], Shim-pack UFLC Shimadzu CBM A system, Shimadzu, Kyoto, Japan; MS, QTRAP System, SCIEX, Framingham, MA). The analytical conditions and the ESI source operation parameters were followed as in the previous study.[59] The qualitative analysis of

the first-order and second-order spectra detected by MS was carried out based on the self-database MWDB (Metware database, Metware, Wuhan, CHINA) and the public database of metabolites information. The quantitation of metabolites was accomplished using multiple reaction monitoring (MRM) triple quadrupole MS.[60]

## Bioinformatics analysis

Counts data from 16s rRNA, metagenomic, metatranscriptomic, and metabolomic profiles were imported into R version 3.5.1. Alpha diversity metrics (richness and diversity) and rarefaction were calculated using the phyloseq package (version 1.26.0). NMDS analysis based on Bray-Curtis dissimilarities was performed using the vegan package (version 2.5-3). Heatmaps were generated using the pheatmap package (version 1.0.10). The A regression analysis via LASSO was done using the glmnet package (version 3.0.2). LASSO regression was fitted with an intercept. All of the analytes with a non-zero β-coefficient in the LASSO models were included. Spearman correlations and p values were calculated using cor and cor.test functions in R and visualized using the corrplot package (version 0.84).

## Data availability

Raw sequence data generated for this study are available in the Sequence Read Archive under BioProject accession PRJNA779750.

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## AUTHOR CONTRIBUTIONS

F.Z. and T.Z. performed the experiments and data analyses and drafted the manuscript. Y.W., Z.X., C.C., and W.Z. assisted in experiments and metagenomics sequencing. A.Y.L. and W.T. collected the human specimens and data. P.K.S.C. and F.K.L.C. provided critical comments. S.C.N. designed and supervised the study.

## DECLARATION OF INTERESTS

FKLC and SCN are the scientific co-founders and sit on the board of Directors of GenieBiome Ltd. SCN has served as an advisory board member for Pfizer, Ferring, Janssen, and Abbvie and a speaker for Ferring, Tillotts, Menarini, Janssen, Abbvie, and Takeda. She has received research grants from Olympus, Ferring, and Abbvie. FKLC has served as an advisor and lecture speaker for Eisai Co. Ltd., AstraZeneca, Pfizer Inc., Takeda Pharmaceutical Co., and Takeda (China) Holdings Co. Ltd. ZX and WT are part-time employee of GenieBiome Ltd. SCN, FKLC, TZ and ZX are inventors on patent held by the CUHK and MagIC that covers use of microorganisms in bodyweight regulation. All other co-authors have no conflict of interest.

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xinn.2022.100304.

## LEAD CONTACT WEBSITE

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Siew C. Ng, https://www.med.cuhk.edu.hk/staff/professor-ng-siew-chien.